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Original Research Article

Abnormalities in Semen Analysis in Male Partners of Infertile Couples at a Tertiary Care Hospital in Garhwal Region of Uttarakhand

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ABSTRACT

Background: Infertility is "failure to achieve a clinical pregnancy after >= 12 months of regular unprotected sexual intercourse". Abnormal semen parameters are found in 50% of the infertile couples. Thus, Semen analysis is usually the first step in evaluating male partner in infertile couples.

Material and methods: This is a 2 years retrospective study including 103 cases. Patient's clinical history was taken and Semen sample was obtained by masturbation in sterile universal container following abstinence of 3-5 days. Using WHO standards, Samples were examined for various physical parameters and sperm motility. Sperms were counted with improved neubauer chamber and stained with Pap stain to observe morphology.

Results: Total 103 male partners of infertile couples were investigated. Abnormal seminal parameters were found in 57.2% with maximum number in 4th decade (41.7%). Abnormal appearances included transparent (5.8%), opaque (1.9%) and redish brown (1.9%). Hypospermia was seen in 23.3% and increased liquefaction time in 1.9% samples. Sperm concentration and total sperms/ejaculate were reduced in 30.7 % and 31.7% cases respectively. Total Sperm motility was below WHO lower reference range in 16.5% cases. However, progressive motility alone was below lower reference range in 25.8% cases. On morphology, maximum defects were seen in the head of spermatozoa. Leucocytospermia was seen in in 9.9 % . Abnormalities in sperm count included oligozoospermia (21.4%), Cryptozoospermia (2.9%) and azoospermia (3.9%). Oligozoospermia was the commonest microscopic abnormality followed by Asthenozoospermia (14.6%).

Conclusion: Semen analysis is the cornerstone of investigating male infertility.

Keywords: Infertility, Semen Analysis

INTRODUCTION

According to WHO, Infertility is "a disease of the reproductive system defined by failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse".[1] It is a social stigma specially in a developing country like ours where child-bearing is strongly recommended and leads to psychological stress and economic loss.[2] Nearly one out of the six couples are affected by primary or secondary infertility in either husband or wife.[3, 4]

Male infertility is the male's inability to impregnate a fertile female. About 40% of infertility cases are due to male factors such as reduced sperm motility, sedentary life style and smoking. [5] Semen quality is also decreased due to Nutritional factors such as Vitamin D deficiency, infections , oxidative stress and increased inflammatory cytokines in seminal fluid which damage sperm DNA.[6,7] Abnormal semen

parameters are found In 50% of the infertile couples .[8]

Thus, Semen analysis after 3 days of abstinence, is usually the first step in evaluating male partner in infertile couples. It analyse the total number of spermatozoa which reflects the efficiency of spermatogenesis by seminiferous tubules and patency of the post testicular structures necessary for sperm transport and ejaculation. The total fluid volume added by various accessory glands reflects their secretory activity.[9,10] The appearance, morphology, concentration, plasma membrane and chromatin integrity help in structural evaluation of sperm while motility, capacitation, and acrosomal reaction lead to functional evaluation.[11]

Our study was conducted to evaluate the abnormalities in seminal patterns among male partners in infertile couples and determine the percentage of abnormalities observed in various parameters assessed.

MATERIAL AND METHODS

This is a 2 years retrospective study from January 2022 to January 2024, including 103 cases of infertility in Government Medical College , Srinagar, Uttarakhand. Patient's clinical history included age, type of infertility, relevant family and medical history. Semen samples were obtained by masturbation in sterile wide mouth plastic universal containers following abstinence of 3-5 days and received within 30 minutes of collection. Urine mixed and spilled samples were excluded from the study. Container was labelled with patient name, registration number, time & date of collection. Using WHO standards, Sample was examined for physical appearance, viscosity, volume and liquefaction time. Wet preparation was made for sperm motility, presence of White Blood Cells or Red Blood Cells. Sperms were counted with the help of improved neubauer chamber and stained with Pap stain to observe the morphology.

Method for sperm motility:

Motility assessments must be made on two different, freshly prepared wet preparations

1. Place a 10 µl well-mixed aliquot onto a clean microscope slide avoiding trapping of air bubbles between the coverslip and slide.
2. Place a 22 mm × 22 mm coverslip carefully over the drop.
3. Assess the freshly made wet preparation under high power (40x) as soon as the contents are settled .
4. Count a total 200 spermatozoa and categorize sperm motility as fast progressively motile, slow progressively motile, non-progressively motile and immotile (grade a, b, c or d) . Record in percentage .

Method of Sperm count:

Ideally the dilution of the ejaculate required to allow sperm concentration to be measured accurately is estimated from the number of spermatozoa observed per high magnification field. We used 1:20 dilution in all samples (50 microL ejaculate +950 microL fixative)

1. Semen is diluted 1:20 with fixative.
2. Charge the improved Neubauer haemocytometer with diluted semen sample and allow it to settle for 10 to 15 minutes
3. Place the chamber under the microscope and count spermatozoa in 4 large corners squares under high power
4. Sperm concentration per ml = sperm count (N) x correction factor for dilution (20) x 1000/ number of squares (4) x volume per square (0.1) = N x 50000/ml
5. Total number of sperms per ejaculate = sperm concentration x volume of semen

Smear examination for sperm morphology:

1. Place a drop of semen on a glass slide, prepare a smear and stain it with Pap stain.
2. At least 200 spermatozoa are counted under oil immersion.
3. Percentage of normal & abnormal spermatozoa is recorded.

The recent WHO criteria (6th edition) were used for interpretation of results of analysis.[9] Values mentioned below represent the accepted lower reference limit of the 5th percentile for parameters measured.

- Appearance – Grey- opalescent
- Volume = >1.5 ml

- Viscosity - Small discrete drops (thread < 2 cm long)
- pH = >7.2
- Sperm concentration = >16 million/ml
- Total sperm count = > 39 million sperm per ejaculate
- Morphology = >4 percent normal
- Vitality = >58% live sperm
- Progressive motility = >30%
- Total (progressive and non-progressive motility) = >42%

RESULTS

Total 103 male partners of infertile couples were investigated in our study with age ranging from 21 to 50 years. One or more abnormal seminal parameters were found in 57.2% cases with maximum number in the 4th decade (41.7%) (Table 1).

Among macroscopic seminal parameters, appearance was normal i.e. opalescent grey in 90.4% cases (figure 1). Abnormal appearances included transparent (5.8%), opaque (1.9%) and redish brown (1.9%).

Decreased seminal volume (Hypospermia) was seen in 23.3% and increased liquefaction time in 1.9% samples (Table 2). Only 101 samples were considered for Sperm concentration (reduced in 30.7 %) and total sperms per ejaculate (reduced in 31.7% cases) as for two samples, the quantity was not sufficient for dilution (Table 3).

Sperm motility and morphology was seen in 97 cases (excluding 4 cases of azoospermia and 2 of insufficient quantity). Total Sperm motility (progressive and Non progressive) was below WHO lower reference range in 16.5% cases. However, progressive motility (PR) alone was below lower reference range in 25.8% cases and 57.7% cases were those with non motile sperms >= 20%. The morphological abnormalities found were head defects (figure 2), middle piece defects (figure 3) and tail defects (figure 4) with maximum defects in the head.

Out of the 101 specimens, Leucocytospermia was seen in 9.9 % (Table 4). Abnormalities in sperm count included 21.4% cases of oligozoospermia, 2.9% of Cryptozoospermia and 3.9% of azoospermia.

There was only 1 case of Necrozoospermia out of 103. Oligozoospermia (21.45%) was the most common microscopic abnormality identified followed by Asthenozoospermia in 14.6% case. Oligoastheno zoospermia was seen in 2.9% cases. There was no case of Teratozoospermia in our study (Table 5).

Table-1: Age distribution (n=103)

Age groups (years)	21- 30	31- 40	41- 50	**Total**
Normal semen analysis	11 (10.7%)	26 (25.3%)	7 (6.8%)	44 (42.8%)
Abnormal semen analysis	5 (4.8%)	43 (41.7%)	11 (10.7%)	59 (57.2%)
Total	16 (15.5%)	69 (67%)	18 (17.5%)	103(100%)

Table-2: Distribution based on Macroscopic features (n=103)

Macroscopic features		Number of patients (%)
Appearance	Opalscent Grey	93 (90.4%)
	Transparent	6 (5.8%)
	Red brown	2 (1.9%)
	Opaque	2 (1.9%)
Seminal volume	Hypospermia (<1.4 ml)	24 (23.3%)
	Normospermia (1.4 ml – 5 ml)	78(75.8%)
	Hyperspermia (> 5 ml)	1 (0.9%)
Liquefaction time	<15 minutes	10 (9..9%)
	15- 30 minutes	86 (85.3%)
	31- 60 minutes	3 (2.9%)
	>60 minutes	2 (1.9%)

Table-3: Distribution based on Sperm concentration and Total sperm count per ejaculate (n = 101)

Sperm concentration in Million / ml	
<16	31 (30.7%)
16 - 66	61 (60.4%)
>66	9(8.9%)
Total sperm count per ejaculate in Million / ml	
<39	32 (31.7%)
39 - 210	63 (62.4 %)
>210	6 (5. 9%)

Table-4: Distribution based on other Microscopic parameter

A. Motility (%) (n = 97)		**Number of patients (%)**
1.Progressive	<30	25 (25.8%)
	30 -55	61 (62.9 %)
	>55	11 (11.3%)
2. Progressive + Non progressive	<42	16 (16.5%)
	42 -64	75 (77.3 %)
	>64	6 (6.2%)
3. Non motile	<20	41(42.3.%)
	>=20	56 (57.7%)
B. Morphology (n = 97)		**Number of patients (%)**
1.Normal Morphology	<4%	0
	>= 4%	97 (100%)
2.Abnormal Morphology		
Head defect	<5 %	60 (61.9%)
	5 -10 %	20 (20.6 %)
	>10 %	17 (17.5 %)
Middle piece defect	<5 %	81 (83.5%)
	5 -10 %	12 (12.4 %)
	>10 %	4 (4.1 %)
Tail defect	<5 %	73 (75.3%)
	5 -10 %	18 (18.6 %)
	>10 %	6 (6.1 %)
C. Pus cells (n = 101)		**Number of patients (%)**
< 1 million/ ml		91 (90.1 %)
>= 1 million/ ml		10 (9.9 %)

Table-5: Distribution of various abnormalities in major semen parameters (n= 103)

Abnormality	**Number**	**Percentage**
Azoospermia	4	3.9
Cryptozoospermia	3	2.9
Oligozoospermia	22	21.4
Necrozoospermia	1	0.9
Asthenozoospermia	15	14.6
Oligo astheno zoospermia	3	2.9

Hypospermia	24	23.3
Hematospermia	2	1.9
Leucocytospermia	10	9.7

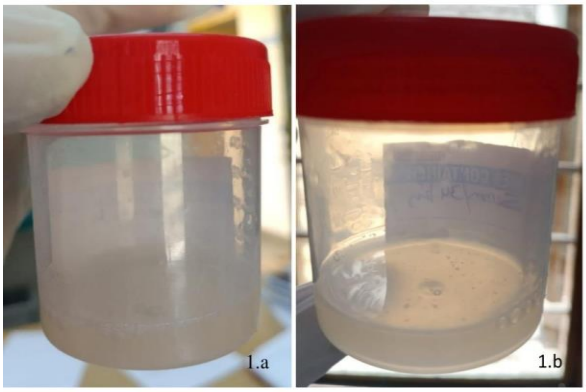

Figure-1: Semen sample in universal container before liquefaction (a) and after liquefaction (b)

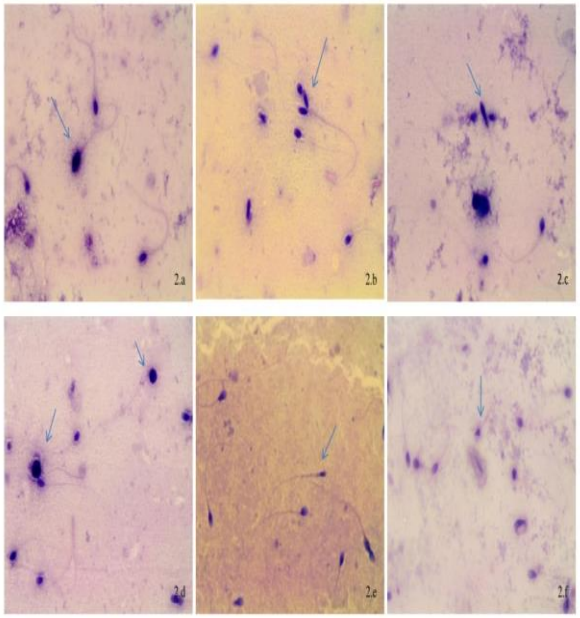

Figure-2: Head defects showing amorphous head (a), double head (b), long head (c), round head (d), small head (e) and conical head (f) [pap ; 100X]

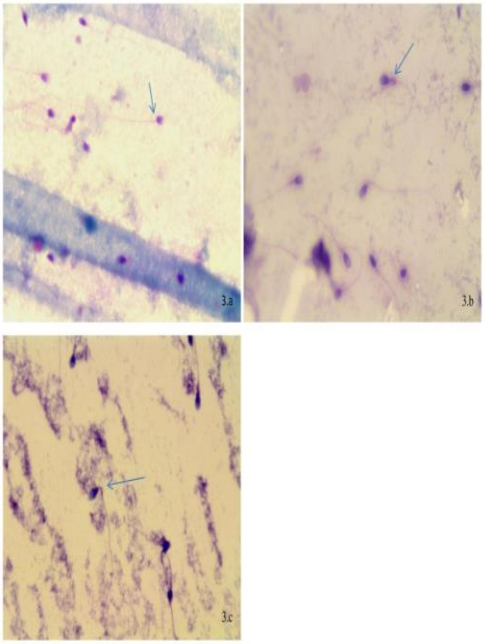

Figure-3: Middle piece defects showing thin middle piece (a), thich middle piece (b), titled neck (c) [Pap ; 100X]

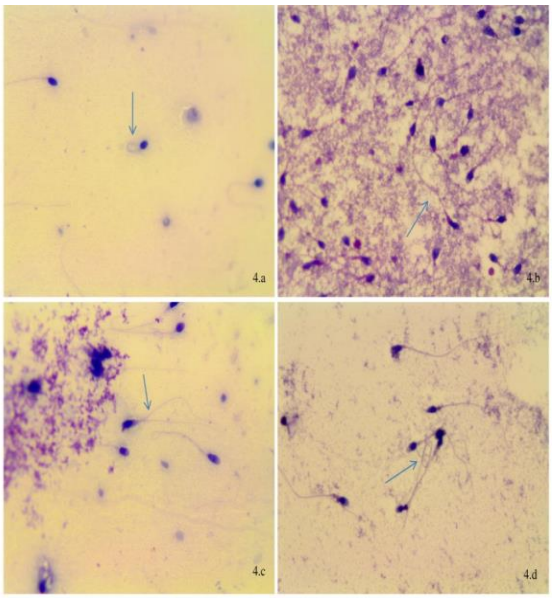

Figure-4: Tail defects showing short tail (a), long tail (b), double tail (c), coiled tail (d) [Pap ; 100X]

DISCUSSION

The very first step in evaluating infertile couples is that if the results of analysis are within WHO reference limits, only testing once is sufficient. However for abnormal results, at least two tests on different occasions along with further hormonal investigations are required.[8]

This study was conducted to determine the abnormalities in semen samples for detection of infertility in 103 male partners of infertile couples. One or more abnormal seminal parameters were found in 57.2% of our cases and were more common after 30 years of age (91.5%). Similar to us, more than 50% cases with abnormal parameters was also reported by Ramya C et al[8] (52.2%) and Jajoo S et al[14] (52%) with maximum cases beyond 30 years in each. However, Jain A et al[reported equal number of cases in both the age groups.

Appearance was opalscent Grey in majority (90.4%). Abnormal appearances included opaque due to excess sperm concentration, transparent due to extremely decreased sperm concentration and redish brown color due to presence of erythrocytes in the semen. This condition is called hematospermia and has varied etiology depending upon patient age (urogenital inflammation and infections in younger patients and serious pathologies like prostate cancer in older men). [15]

Decreased seminal volume (Hypospermia) was seen in 23.3% and hyperspermia in 0.9% cases. Mahdi et al[4] showed 24.5% cases of hypospermia and 1.5% hyperspermia which was nearly similar to us , while studies by Jain A et al[2] ,Tandel et al[3] and Bhaduri N et al[10] showed hypospermia in 28%, 16 % and 7.5% cases respectively. Hypospermia may be due abnormalities in secretions by accessory sex glands such as seminal vesical, defect in transport such as ejaculatory duct obstruction , congenital bilateral absence of the vas deferens where the seminal vesicles have not developed, retrograde ejaculation, or less duration of abstinence. [4, 16]

Normal Liquefaction time of the semen is 15–30 minutes. The process is regulated by prostatic secretions containing proteolytic enzymes (lysozyme, α-amylase, and β-glucuronidase) and prostate specifc antigen (a trypsin-like protease) that cleaves the semenogelin proteins.[9] Increased liquefaction time(> 60 minutes) was seen in 1.9% of our samples. In study by Ramya C et al.[8] 5.03% cases had increased liquefaction time. This may be due to altered prostate specifc antigen because of congenital or acquired factors such as prostatitis .

Sperm concentration is the quantitative marker of spermatogenesis, while sperm motility and morphology are qualitative parameters. Out of the total 101 samples considered for Sperm concentration, it was below WHO lower reference limit in 30.7 % cases. There was a wide variation in sperm concentration abnormalities in various studies. [2, 3, 8] These may be due to difference in sample size, method of semen collection and time of the study as WHO lower reference limit for sperm concentration has changed over years. Also, sperm count and quality is declining over time because of exposure to various chemicals which lead to hormonal imbalance.[17]

Total motility includes progressive and non-progressive motility where Progressive motility is the spermatozoa moving actively in large circle or linearly, regardless of the speed and Non-progressive motility denotes all other patterns of motility without progression.[18] Reduction in sperm motility is called asthenozoospermia and can be due to congenital or acquired causes. It was found in 16.5% cases in our study which was near to findings by Mahdi et al[4] (13%) and showed variation from Ramya C et al[8] (23.2 %), Bhaduri N et al[10] (4.4%) and Kalavathi et al [19] (1.2%cases) .

There was no case of Teratozoospermia in our study. This can be because the WHO reference limit for normal sperm morphology is >=4%. Almost all our cases had normal morphology >4%. However, various degrees of abnormalitie were seen in head, middle piece and tail. These mixed morphological defects can be due to defective spermatogenesis or epididymal infammation.[9]

Leucocytospermia was seen in 9.9 % cases which can be due to infection or inflammation in urogenital tract (testes, prostate, seminal vesicles, bulbo urethral glands). Tandel et al[3] reported a much higher percentage (52%).

Abnormalities in sperm count included oligozoospermia (21.4%), Cryptozoospermia (2.9%) and azoospermia (3.9%). Studies by Tandel et al[3], Ramya C et al[8], Kalavathi et al[19] and Mahdi et al[4] showed 35%, 32.1% ,24.8% and 12 % cases of oligozoospermia respectively. Oligozoospermia (21.45%) was the most common microscopic abnormality identified followed by Asthenozoospermia (14.6%). Oligozoospermia was also the most common abnormality reported by Ramya

C et al[8] (32.1%) and Bhaduri N et al[10] (19.9%) while Asthenozoospermia was most common abnormal parameter in study by Jain A et al[2].

A comparison of the parameters with other studies has been shown in Table 6.

Table 6: Comparison of the parameters of our study with other studies

Age (years)	**Ramya C et al[8] (n=83)**	**Jajoo S et al[14] (n=100)**	**Jain A et al[2] (n=50)**	**Our study (n=59)**
< 30	46.9%)	48%	50%	8.5%
>30	53.1%	52 %	50%	91.5%
Semen volume	**Mahdi et al[4] (n=1000)**	**Tandel et al[3] (n=200)**	**Jain A et al[2] (n=50)**	**Our study (n=103)**
Hypospermia	24.5%	16%	28.0%	23.3%
Hyperspermia	1.5%		2%	0.9%
Sperm concentration	**Ramya C et al[8] (n=159)**	**Tandel et al[3] (n=200)**	**Jain A et al[2] (n=50)**	**Our study (n=101)**
Below WHO lower reference limit for the respective period	39.6%	4%	70%	30.7 %
Total Motility	**Ramya C et al[8] (n=159)**	**Kalavathi et al[19] (n=250)**	**Mahdi et al[4] (n=1000)**	**Our study (n=97)**
Asthenozoospermia	23.2%	1.2%	13%	16.5%

CONCLUSION

Despite the limitations and pitfalls of routine semen analysis, it continues to remain the cornerstone of investigating male infertility. It provides a guideline to the clinicians to plan further diagnostic investigations based on the abnormalities detected on routine examination.

Limitations of Study

The Semen parameters assessed during routine evaluation are susceptible to preanalytical and analytical variations along with interobserver variability. Therefore it is a safe practice to always have a second evaluation. Unfotunately, not all patients are cooperative in this aspect.

The microscopic examination of semen which is done routinely, is incapable of giving any information regarding the functional integrity of the spermatozoa like ultrastructural defects and DNA fragmentation. Whether the spermatozoa has the ability to bind to Zona pellucida or to fertilize the egg can not be evaluated by routine examination. Therefore, the WHO manual has now suggested advanced tests to assess the competence of the spermatozoa which are essential for conception.

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